PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

VOSSIUS & PARTNER
Siebertstrasse 4
D-81675 München
ALLEMAGNE

EINGEGANGEN
Vossius & Partner
22. Aug. 2005
Frist bearb.:

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 22.08.2005
Applicant's or agent's file reference G 2593 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/003921	14.04.2004	15.04.2003

Applicant
MAX-PLANCK-GESELLSCHAFT ZUR F\RDERUNG DER.. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Bröcker-Tazelaar, G Tel. +31 70 340-2103

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G 2593 PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/003921	International filing date *(day/month/year)* 14.04.2004	Priority date *(day/month/year)* 15.04.2003
International Patent Classification (IPC) or national classification and IPC C12N15/10, C12N15/66, C12P19/34, C12Q1/68		
Applicant MAX-PLANCK-GESELLSCHAFT ZUR F\RDERUNG DER.. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 1-3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.02.2005	22.08.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Hornig, H Telephone No. +31 70 340-2620

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/003921

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-29 as originally filed

Sequence listings part of the description, Pages

1-4 as originally filed

Claims, Numbers

1-21 received on 15.02.2005 with letter of 15.02.2005

Drawings, Sheets

1/4-4/4 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 22
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.

PCT/EP2004/003921

Re Item I.

1.1 The amended claims 1-21 filed with letter dated 15.02.2005 and received on 15.02.2005 are allowable according to Art. 34(2)(b) PCT. The basis of the report issues on the claims 1-21 as amended according to Rule 70.2 PCT.

Re Item V.

1 The following documents are referred to in this communication:

D1 : HAI-BAO CHEN ET AL: "A NEW METHOD FOR THE SYNTHESIS OF A STRUCTURAL GENE" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 18, no. 4, 25 February 1990 (1990-02-25), pages 871-878, XP000099685 ISSN: 0305-1048

D2 : WO 97/42330 A (APOLLON INC) 13 November 1997 (1997-11-13)

D3 : WO 00/29616 A (PERKIN ELMER CORP) 25 May 2000 (2000-05-25)

D4 : STEFAN KALUZ ET AL. "ENZYMATICALLY PRODUCED COMPOSITE PRIMERS: AN APPLICATION OF T4 RNA LIGASE-COUPLED PRIMERS TO PCR" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, vol. 19, no. 2, August 1995 (1995-08), page 182,184,186, XP001160946 ISSN: 0736-6205

D5 : WO 89/11211 A (BIOTECHNOLOG FORSCHUNG GMBH) 30 November 1989 (1989-11-30)

2 INDEPENDENT CLAIM 1

2.1 Document D1 discloses a method of synthesizing a structural gene or gene fragment, consisting of the first synthesis of a single-stranded DNA (ssDNA).

A plus-stranded DNA of the target gene was generally assembled by a stepwise or one step T4 DNA ligase reaction of six oligonucleotides (A, *pB, *pC, *pD, *pE and *pF) of 30 - 71 nucleotides long in the presence of two terminal complementary oligonucleotides (Ab' and eF') and three short interfragment complementary

oligonucleotides (bc, cd and de). After purification, the synthetic ssDNA was inserted into a cloning vector, pWR13.

Document D2 discloses the synthesis of composite nucleic acid molecules - by chain reaction cloning using nucleic acid molecules, a bridging oligo-nucleotide and a thermostable ligase. The composite nucleic acid molecule (NAM) was synthesised by ligating a first NAM to a second NAM, comprising: (a) maintaining a first NAM and a second NAM at a denaturing temperature; (b) forming a mixture comprising the first NAM and the second NAM with a bridging oligonucleotide (ON) and a thermostable ligase (TL), where the bridging ON comprises: (I) a 10-40 nucleotide sequence at its 5' end which is complementary to a nucleotide sequence on the 3' end of a strand of the first NAM; (ii) a 10-40 nucleotide sequence at its 3' end which is complementary to a nucleotide sequence on the 5' end of a strand of the second NAM; where at annealing temperature, the bridging ON hybridises to sequences on the sense strand of both the first and second NAM or on the antisense strand of both the first and second NAM; © maintaining the mixture at an annealing temperature, whereby the bridging ON hybridises to sequences of both the first and second NAM and the TL ligates either the 3' end of the first NAM to the 5' end of the second NAM, or the 5' end of the first NAM to the 3' end of the second NAM to form a first strand of the composite NAM hybridised to a bridging ON; (d) maintaining the mixture at a denaturing temperature, whereby the bridging ON dissociates from the first strand of the composite NAM; and (e) maintaining the mixture at an annealing temperature, whereby the first strand of the composite NAM hybridises to complementary strands of the first NAM and the second NAM, where the TL ligates the end of the complementary strand of the first NAM to the end of the complementary strand of second NAM to form a second strand of the composite NAM.

Document D3 discloses Ligation assembly and detection of polynucleotides on solid support allowing the creation of a single stranded polynucleotide of 50 to 500 nucleotides. Said method comprises: (a) annealing at least one bridging oligonucleotide and two or more assembly oligonucleotides such that a ligatable nick site is formed between adjacent assembly oligonucleotides, where one of the assembly oligonucleotides is immobilised to a solid support; (b) ligating the nick sites to form an immobilised single-stranded ligation product:

2.1.1 In the light of the prior art documents D1-D3 the present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 is new in the sense of Article 33(2) PCT.

2.2 Document D4, which is considered to represent the most relevant state of the art to the subject matter of claim 1, discloses enzymatically produced composite primers consisting of two parts: a gene-specific segment, which is unique for each primer, and an auxiliary segment (adaptor), which is usually the same for any particular application. The gene-specific and adaptor of a composite primer have clearly distinct function, and the problem of repeated incorporation of the same adaptor to different primers could be alleviated by synthesizing the adaptor segment separately and coupling it enzymatically to the gene-specific primer before e.g. PCR amplification. D4 describes the ligation of an 3' end of an adaptor to the 5'-end of a phosphorylated 5'-end of a second gene specific oligonucleotide and its use in PCR.

2.2.1 The subject-matter of independent claim 1 differs from the disclosure of D4 in that D4 does not disclose the technical feature of annealing a single stranded adaptor oligonucleotide which is complementary to the first respectively second oligonucleotide, in linking said two oligonucleotides.

2.2.2 The problem to be solved by the present invention may therefore be regarded as an alternative method of producing a single-stranded nucleic acid molecule from oligonucleotides.

2.2.3 D5 describes an oligonucleotide bank contains all conceivable (i.e. 4 to the power 6, 7, 8 or 9) different hexameric, heptameric, octameric or nonameric oligonucleotides. To sequence DNA, a single-stranded primer attachment site of known sequence is selected and to this site 2 immediately adjacent, hexa-, hepta- or octameric oligonucleotides are hybridised. Optional a third similar oligonucleotide is simultaneously, or subsequently, hybridised immediately adjacent to 1 of the other 2, and the oligomers ligated to form a primer. Particularly, the 2 oligomers are ligated to form a pre-primer, then the third

hybridised and ligated with the pre-primer. The oligonucleotides are phosphorylated at the 5'-end where this end is to be ligated to a neighbouring oligonucleotide using T4 ligase. After ligation and before sequencing, any non-ligated oligonucleotide is removed by heat-treatment.

2.2.4 The combination of the features of the dependent claims 2-21 are neither known from, nor rendered obvious by, the available prior art. In the light of the prior art documents D4 and D5, the present application does meet the criteria of Article 33(1) PCT, because the subject matter of claim 1 appears to comprise an inventive step in the sense of Article 33(3)PCT.

Re Item VI.

D6 : BORODINA TATIANA A ET AL: "Ligation-based synthesis of oligonucleotides with block structure." ANALYTICAL BIOCHEMISTRY, vol. 318, no. 2, 15 July 2003 (2003-07-15), pages 309-313, XP002302313 ISSN: 0003-2697

D7 : WO 03/106637 A (DATASCOPE INVEST CORP ; ZAK S ARIEH (US); GETTS LORI (US); GETTS ROBER) 24 December 2003 (2003-12-24)

D8 : EP1327682 A (BIOSPRING GES FUER BIOTECHNOLO) 16 July 2003 (2003-07-16)

Re Item VIII.

1. Clarity (Art. 6 PCT)

1.1 The expression **"optionally"** in claims 1(a) and (b) has been considered as having no limiting effect on the scope of said claims (Art. 6 PCT).